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# Processed elderberry (*Sambucus nigra* L.) products: A beneficial or harmful food alternative?

Mateja Senica*, Franci Stampar, Robert Veberic, Maja Mikulic-Petkovsek

*Department of Agronomy, Chair for Fruit Growing, Viticulture and Vegetable Growing, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia*

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ABSTRACT

Elderberry fruit and its food products are not only a rich source of phenolics, boosting their antioxidant activity, but also contain harmful cyanogenic glycosides. In order to assess the potential positive/negative effects of consuming elderberry-based products their biochemical profile and levels of individual compounds were identified with the aid of high-performance liquid chromatography (HPLC) coupled with mass spectrophotometry (MS). Cyanidin-3-sambubioside and cyanidin-3-glucoside were the prevalent compounds among phenolics and sambunigrin among cyanogenic glycosides in all analyzed elderberry products. Processing considerably affects the content of elderberry phenolics and cyanogenic glycosides. The levels of phenolics decreased from 958 mg/kg in unprocessed control berries to 343 mg/kg in elderberry liqueur, 337 mg/kg in spread, 162 mg/kg in tea and 114 mg/kg in elderberry juice. Higher temperatures not only reduced the content of beneficial compounds, but also decreased the levels of harmful cyanogenic glycosides for 44% in elderberry juice, for 80% in tea and for as much as 96% in elderberry liqueur and spread.



## 1. Introduction

Black elder or elderberry (*Sambucus nigra* L.) is a deciduous, tree-like shrub, widespread in almost every continent of the world. It usually blooms from May to July and the berries ripen from August to late September. The creamy-white flowers and shiny, purplish-black berries have been used for centuries for medicinal purposes and are traditionally consumed to prevent or diminish the effects of several diseases (Schmitzer, Veberic, & Stampar, 2012). The potent healing effects of elderberry has been linked to its many phytochemicals, such as flavonoids, phenolic acids, different organic acids, major and trace elements (K, Ca, P, Mg, …) and vitamins. A similar phytochemical profile has been studied in detail in *Vaccinium* berries (Milivojevic et al., 2012). Due to elderberry's beneficial properties numerous food and beverage products have been generated and offered to the market (Diviš, Pořízka, Vespalcová, Matějíček, & Kaplan, 2015; Cejpek, Maloušková, Konečny, & Velíšek, 2009; Mikulic-Petkovsek, Samoticha, Eler, Stampar, & Veberic, 2015a; Veberic, Jakopic, Stampar, & Schmitzer, 2009; Vlachojannis, Zimmermann, & Chrubasik-Hausmann, 2015; Jiménéz et al., 2014). Especially in Europe, elder flowers and fruit serve as an alternative source in food industry to produce pies, jellies, jams, ice creams, yogurts and different beverages, such as wine, tea, liqueur and juice (Schmitzer, Veberic, Slatnar, & Stampar, 2010; Vlachojannis et al., 2015). The purplish-black color of *S. nigra* fruit has been linked to the levels of different anthocyanins, which represent the highest part of elderberry fruit phenolics (Mikulic-Petkovsek et al., 2014).

Contrary, black elderberries not only have beneficial and antioxidant properties; its leaves, seeds, bark and unripe berries also accumulate potentially toxic compounds (Dellagreca et al., 2003; Skidmore-Roth, 2005). Black elder contains substantial amounts of a cyanogenic glycoside, sambunigrin, which is considered highly toxic due to its decomposition to chemically reactive hydrogen cyanide (Bromley, Hughes, Leong, & Buckley, 2005). Ingestion of elderberry parts in larger doses may cause gastrointestinal disorders, such as nausea, vomiting, weakness and dizziness (Jiménez et al., 2014). The presence of cyanogenic glycosides in elderberry seeds may inhibit their microbial decay, prolonging their survival in adverse circumstances (Losey, Stenholm, Whereat-Phillips, & Vallianatos, 2003).

Because of gastrointestinal disorders caused by cyanogenic glycosides elderberry processed products are consumed in much

* Corresponding author.
*E-mail address:* mateja.voncina@bf.uni-lj.si (M. Senica).

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greater proportions than fresh fruit (Cejpek et al., 2009). Thermal processing namely significantly decreases the levels of these harmful compounds (Turner & Sczawinski, 1991; Onyeike & Omubo-Dede, 2002). However, most bioactive compounds, such as anthocyanins, are relatively unstable and decompose when subjected to heat (Symonowicz, Sykuła-Zajac, Łodyaga-Chruścińska, Rumora, & Straukas, 2012; Wang & Xu, 2007). Heat processed foods are thus considered to possess lower health promoting capacity than the corresponding fresh analogue (Choi, Lee, Chun, Lee, & Lee, 2006).

In recent years several studies have been conducted in order to determine the contents of beneficial phenolic compounds (Mikulic-Petkovsek, Ivancic, Todorovic, Veberic, & Stampar, 2015b; Veberic et al., 2009; Wu, Gu, Prior, & McKay, 2004) and harmful cyanogenic glycosides in different parts of elderberry (Bromley et al., 2005; Losey et al., 2003; Skidmore-Roth, 2005). To our knowledge, no research has focused on analyzing the contents of selected beneficial and adverse compounds in different elderberry products, commonly included in human diet. In order to set recommendations for safe consumption of elderberry products it is essential to determine the extent of decomposition of cyanogenic glycosides in various elderberry products and on the other hand establish the potency of these products in terms of phenolic composition. The ratio between these two harmful/beneficial groups of compounds may ascertain the benefits of elderberry products consumption. So, the aim of the present study was the identification of phenolics and cyanogenic glycosides in several different elderberry food products (juice, liqueur, tea and spread) favored by the consumers.

## 2. Material and methods

### 2.1. Fruit material

Elderberry fruit was collected from wild *S. nigra* population population at Vojsko (Slovenia) (location: latitude 46°38′23.7″ N, longitude 24°45′42.75″ E, 1100 m altitude) in autumn 2015 (17.9.2015) in optimum fruit maturity. All samples were collected at the same location, from several shrubs. Stalks were removed and fruits were immediately processed into different products or analyzed to serve as the control.

### 2.2. Preparation of four different elderberry products (liqueur, juice, tea and spread)

Preparations of different elderberry products were based on most commonly used local recipes. Elder berries were removed from stems prior to processing. Elderberry liqueur was prepared from 5 g of ripe berries and 6 mL double distilled water boiled for 5 min 10 mL of alcohol were added to the mixture and the blend was stirred and filtered through a polyamide filter into vials. The preparation of liqueur was a model approach to traditional way of extraction. Elderberry juice was extracted from 100 g of ripe berries, which were crushed in a plastic bag. The obtained juice was poured into a beaker and 50 mL of fresh elderberry juice was slowly heated for 30 min and left boiling for additional 5 min. Elderberry tea was prepared from 1.5 g (1 teaspoon) of dried elderberries infused in 100 mL boiling water for 10 min. Dry elderberry fruits, used for infusion were firstly exposed to 105 °C for 3 days (14% dry matter). Juice and tea were also filtered through polyamide filters. For the preparation of elderberry spread 50 g of fruits were topped with 6 mL of double distilled water and heated to a boiling point. Heat was reduced to 70 °C and the spread was cooked for an hour. 3 g of spread was extracted with 5 mL of 700 mL/L MeOH with 300 mL/L $H_2O$ and transferred to a vial through a polyamide filter. Each elderberry product was prepared in 7 repetitions.

### 2.3. Extraction of cyanogenic glycosides and phenolics from the control sample

For the biochemical analysis of control sample elderberries were crushed in a mortar and 1 g of elderberry paste was put into a 10 mL screw-cap tube. Solid sample was extracted with 5 mL of 700 mL/L MeOH with 300 mL/L $H_2O$ for 30 min at 30 °C according to the method of Oomah, Mazza, and Kenaschuk (1992). The sample was centrifuged for 7 min at 4 °C and 12,522 g and the supernatant was filtered through a polyamide filter (Macherey–Nagel; Düren, Germany) into vials prior to HPLC and MS analysis. Control treatment was performed in 7 repetitions.

### 2.4. HPLC–DAD coupled with $MS^n$ analysis of phenolic compounds

Individual phenolics were identified on an Accela HPLC system (Thermo Scientific, San Jose, CA) with a diode array detector (DAD) controlled by a CromQuest 4.0 chromatography workstation software. Phenolic compounds were analyzed at 280 nm, 350 nm and 530 nm. The column used was a Gemini $C_{18}$ (150 × 4.6 mm 3 μm; Phenomenex, Torrance, USA), operated at 25 °C. Mobile phase A was 1 mL formic acid with 30 mL of acetonitrile (ACN) topped up with double distilled water to obtain 1 L of solution; mobile phase B was 1 mL of formic acid with 30 mL of double distilled water topped up to 1 L with ACN. Samples were eluted according to the linear gradient described by (Wang, Zheng, & Galletta, 2002). The injection volume was 20 μl and flow rate maintained at 0.6 mL/min.

All phenolic compounds in elderberry products were identified with a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative ion mode. The analyses were carried out using full scan data-dependent $MS^n$ scanning from *m/z* 110 to 1600. The injection volume was 10 μL and the flow rate maintained at 0.6 mL/min. Other technical characteristics were described in study of Mikulic-Petkovsek et al. (2015b). Contents of selected phenolics were expressed in mg per kilogram of elderberries.

### 2.5. MS detection of cyanogenic glycosides

Separation of cyanogenic glycosides was performed on a HYPERSIL GOLD aQ column (Thermo Scientific) at 25 °C and flow rate 0.8 mL/min. The sample injection volume was 20 μL. Mobile phase A was 30 mL of methanol topped up with 970 mL of double distilled water to obtain 1 L; mobile phase B was 30 mL of double distilled water topped up with 970 mL of methanol to 1 L, with gradient elution: 0–1 min, 80% A; 1–8 min, 80–0% A; 8–10 min, 0% A; 10–14 min, 80% A.

The presence and the contents of cyanogenic glycosides was identified and quantified on a TSQ Quantum Access Max quadrupole mass spectrometer. The MS instrument was operated using an (ESI) source in positive ion mode. The ESI parameters were as follows: capillary temperature 275 °C, corona voltage 4.7 kV, sheat gas 60 L/h, auxiliary gas 10 L/h. Mass spectra were scanned in range from *m/z* 70 to 650. Collision-induced dissociation was achieved using argon as the collision gas in the collision cell. Cyanogenic glycosides were analyzed in selected reaction monitoring (SRM) mode and by comparison with MS data of standards. Data acquisition was performed using Xcalibur 2.2.Software. Contents of sambunigrin were expressed in mg per kilogram of elderberries.

### 2.6. Chemicals

The following standards were used for quantification of some phenolics: quercetin-3-*O*-glucoside, (+) catechin and *p*-coumaric acid from Fluka Chemie (Buch, Switzerland). Other commercially

available standards for phenolics and cyanogenic glycosides (prunasin), were obtained from Sigma–Aldrich (Steinheim, Germany). Methanol was purchased from Sigma–Aldrich. Acetonitrile and formic acid from Fluka Chemie GmBH. Double distilled water was produced with a Millipore purification system (Millipore, Bedford, MA, USA) and used to prepare all aqueous solutions.

### 2.7. Statistical analysis

All statistical analyses were performed with R-Commander. Results are presented as mean ± standard deviation of seven replications. Significant differences among different elderberry preparations were calculated by one-way analysis of variance (ANOVA). The differences in the content levels of each individual component were estimated with Duncan's multiple range test and were considered significant at $p < 0.05$.

## 3. Results and discussion

The content of bioactive compounds and cyanogenic glycosides of elderberry products were expressed in mg/kg (Table 2). To adequately compare different beverages (tea, juice and liqueur) with food (elderberry spread) all contents were calculated according to the initial weight of fresh fruits. Furthermore, in Fig. 1 were separately presented harmful and harmless compounds for beverages and expressed in mg/L.

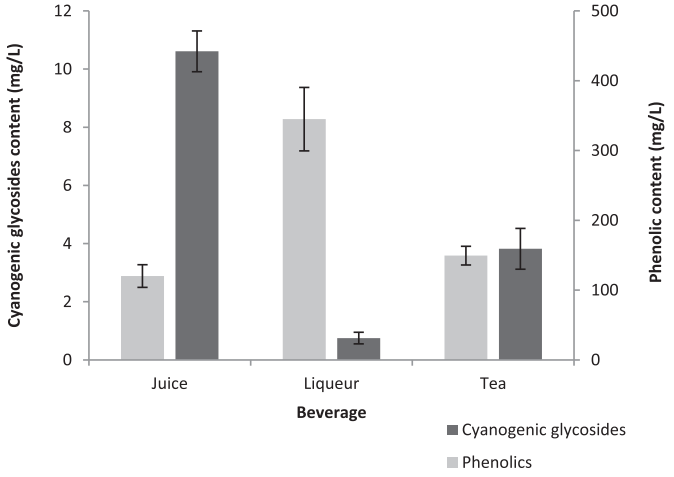


**Fig. 1.** The content of phenolic compounds and sambunigrin (cyanogenic glycoside) (mg/L) in different elderberry beverages; n = 7.

Phenolics are known for their positive effect on human health (Symonowicz et al., 2012). Twenty-eight (28) different individual phenolics and one cyanogenic glycoside have been identified in Table 1. Identification of individual metabolites in different elderberry products is summarized in Table 2. Individual phenolic compounds have been grouped into the following phenolic groups: hydroxycinnamic acids, flavanols, flavonols (mainly quercetin derivatives), flavanones (naringenin derivatives) and anthocyanins. The latter represented the major part of phenolics in all elderberry products analyzed in the study and were predominately responsible for berry color. Five (5) different anthocyanins have been detected in all analyzed elderberry products. Cyanidin 3-*O*-sambubioside (100 mg/kg in elderberry liqueur, 77 mg/kg in spread, 48 mg/kg in juice and 21 mg/kg in tea) and cyanidin-3-*O*-glucoside (88 mg/kg in liqueur, 42 mg/kg in juice and 35 mg/kg in tea) were most prevalent (Table 2). Vlachojannis et al. (2015) and Mikulic-Petkovsek et al., (2014) also reported these two anthocyanins as the major pigments in elderberry fruit. In addition to cyanidin 3-*O*-sambubioside and cyanidin-3-*O*-glucoside cyanidin-3-sambubiosyl-5-glucoside, cyanidin-3-*O*-rutinoside and cyanidin-3,5-*O*-diglucoside have additionally been identified in elderberry products but in much smaller levels. Cejpek et al. (2009) determined that cyanidin-3-*O*-sambubioside comprised 51% total anthocyanins (TA) and cyanidin-3-glucoside 40% TA in elder berries. Correspondingly, cyanidin-3-glucoside accounted for 39% TA and cyanidin-3-sambubioside for 44% TA in elder products analyzed in the present study. Contents of both prevalent anthocyanins were at least for 3 times lower in products than contents in starting material, 328.1 mg/kg for cyanidin 3-*O*-sambubioside and 288.7 mg/kg for cyanidin-3-*O*-glucoside. The stability of anthocyanins is affected by several factors (Chrubasik, Li, & Chrubasik, 2010; Symonowicz et al., 2012; Wang & Xu, 2007). In addition to pH level, light, oxygen, selected enzymes and other factors, heat treatment crucially diminishes the level of anthocyanins in plant samples. The duration and temperature significantly influences anthocyanin degradation. Higher stability of anthocyanins has been achieved by using lower temperatures and shorter heating time during processing (Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006; Wang & Xu, 2007; Dai & Mumper, 2010). Moreover, Cejpek et al. (2009) reported that different extraction temperatures modify anthocyanin release from the cells and temperatures above 60 °C have been confirmed to decrease anthocyanin levels in extracts (Wang & Xu, 2007). Thermal degradation of anthocyanins occurred due to hydrolysis and hydrolization of selected compounds produced into chalchone, which is responsible for brown colour in different products containing anthocyanins (Laleh et al., 2006), what we also observed in our elderberry products, especially in juice. In the present study elderberry liqueur retained the highest anthocyanin content of all elderberry preparations (238 mg/kg). Nevertheless, the content of anthocyanins in elderberry liqueur was 3 fold lower compared to the control (733 mg/kg). High anthocyanin levels in the liqueur can be ascribed to short heating duration as the products was heated to 100 °C and boiled for 5 min. A slightly lower anthocyanin content has been measured in elderberry spread (196 mg/kg), which was initially heated to the boiling point and cooked at 70 °C for 60 min. A 7 fold lower anthocyanin content has been determined in elderberry juice compared to the control (98 mg/kg) although it was prepared by slow heating at 70 °C for 30 min and quickly boiled at the last step (5 min). Therefore, it seems that anthocyanin decline of elderberry juice must be triggered by additional factors. Correspondingly, Wang and Xu (2007) and Dai and Mumper (2010) reported that the change of oxidation of phenolics as a consequence of extraction time and high temperature increase accelerates the rate of chemical reactions and consequently contributes to lower anthocyanins contents and change of colour in fruit juices. Last of all, elderberry tea contained the smallest amount of anthocyanins (78 mg/kg). Dry elderberry fruits were used to prepare the infusion and in the first step elder berries were exposed to 105 °C for 3 days. This process decreased anthocyanin levels for approx. 17% (data not shown). Dried fruits were then infused with boiling water and left for 10 min. Juice and tea, non-alcoholic beverages showed the lowest contents of anthocyanins. Another reason is also anthocyanins extractability, as their extraction better performed in organic than water solvents (Laleh et al., 2006; Wang & Xu, 2007; Dai & Mumper, 2010).

In addition to anthocyanins, flavonols represented the second major phenolic group in all analyzed elderberry products. This group represented up to 12% of all analyzed phenolics. Quercetin-3-*O*-rutinoside was the major compound and was characteristic for all elderberry products (from 2 to 65 mg/kg). The highest level of this flavonol has been measured in elderberry spread (65.0 mg/kg), followed by the liqueur (38 mg/kg). The smallest proportion of

**Table 1**
HPLC-MS identification of phenolic compounds in elderberry products in negative and positive ionization mode and cyanogenic glycosides in elderberry products in positive ionization mode.

| Phenolics | $\lambda_{max}$ (nm) | MS *m/z* | $MS^2$ | $MS^3$ | Control | Juice | Liqueur | Tea | Spread |
|---|---|---|---|---|---|---|---|---|---|
| **Hydroxycinnamic acids** | | | | | | | | | |
| Chlorogenic acid (*trans*-5-caffeoylquinic acid) | 234, 328 | 353 | 191, 179 | 127, 93, 85 | + | + | + | + | + |
| Neochlorogenic acid (3-caffeoylquinic acid) | 234, 326 | 353 | 191, 179, 135 | 173, 127, 85 | + | + | + | + | + |
| *p*-Coumaric acid | 310 | 163 | 119 | 128 | + | + | + | | |
| *p*-Coumaric acid hexoside | 316, 287, 234 | 371 | 325, 163 | 119, 128 | | | | + | + |
| 3-*p*-Coumaoylquinic acid | 310, 234 | 337 | 163, 191, 173 | | + | + | + | | + |
| 4-*p*-Coumaoylquinic acid | 310, 234 | 337 | 173 | | + | + | + | | + |
| 5-*p*-Coumaoylquinic acid | 310, 234 | 337 | 191, 173, 163 | | + | + | + | + | + |
| 4-Caffeoylquinic acid | 328, 234 | 353 | 173, 179 | | + | + | + | + | + |
| *cis*-5-Caffeoylquinic acid | 328, 234 | 353 | 191 | 179 | + | + | + | + | + |
| Dicaffeoylquinic acid | 247, 316 | 515 | 353 | 179, 173 | + | + | + | + | + |
| **Flavanols** | | | | | | | | | |
| (+) Catechin | 234, 279 | 289 | 245 | 205, 179 | + | + | + | | + |
| (−) Epicatechin | 234, 279 | 289 | 245 | 205, 179 | + | + | + | | + |
| **Flavonols** | | | | | | | | | |
| Quercetin-3-*O*-glucoside | 255,355 | 463 | 301 | 179, 151 | + | + | + | + | + |
| Quercetin-3-*O*-rutinoside | 255,355 | 609 | 301 | 179, 151 | + | + | + | + | + |
| Quercetin-acetylglucoside | 255, 355 | 505 | 463 | 301 | + | + | + | + | + |
| Quercetin-hexoside pentoside | 254, 358 | 595 | 301 | | + | + | + | + | + |
| Kaempferol-3-rutinoside | 266, 348 | 447 | 285 | 257, 229 | + | | + | | + |
| Isorhamnetin-3-rutinoside | 255, 352 | 477 | 315 | 300, 271, 151 | + | + | + | + | + |
| **Flavanones** | | | | | | | | | |
| Naringenin hexoside | 283, 340 | 433 | 271 | 151 | + | | + | | + |
| **Anthocyanins** | | | | | | | | | |
| Cyanidin-3-*O*-glucoside | 280, 518 | 449 | 287 | | + | + | + | + | + |
| Cyanidin-3-*O*-rutinoside | 280, 517 | 595 | 449 | 287 | + | + | + | + | + |
| Cyanidin-3-*O*-sambubioside | 280, 520 | 581 | 287 | | + | + | + | + | + |
| Cyanidin-3,5-*O*-diglucoside | 280, 518 | 611 | 581 | 287 | + | + | + | + | + |
| Cyanidin-3-sambubiosyl-5-glucoside | 280, 518 | 743 | 581 | 449, 287 | + | + | + | + | + |
| **Cyanogenic glycosides** | | | | | | | | | |
| Sambunigrin | 278 | 318 | 185, 128 | | + | + | + | + | + |

quercetin-3-*O*-rutinoside has been determined in elderberry juice (2 mg/kg). Quercetin-3-*O*-glucoside was the second prevalent flavonol detected in products made from elderberry fruits (from 0.2 to 10 mg/kg). All other analyzed flavonols were represented in smaller contents (Table 2). Content levels of quercetin derivatives in elderberry fruit were comparable to the study of Mikulic-Petkovsek et al., (2015b). Quercetin-3-acetylglucoside, quercetin-hexoside pentoside 2 and isorhamnetin-rutinoside could not be detected in elderberry tea and isorhamnetin-rutinoside was not present in the juice. Chemical changes, induced by thermal processing, alter the flavonol composition of different elderberry products. Zorić, Dragović-Uzelac, Pedisić, Kurtanjek, and Garofulić (2014) reported, that quercetin glycosides are the most thermally sensitive compounds from phenolics and was the reason of flavonol decline in our study. The content of flavonoids declines with increasing both the duration of heating and the heating temperature (Choi et al., 2006). A potential reason for variations in flavonol contents was the use of different analytical solvents for different elderberry products. We must take into account that organic solvent seems to be the best for extraction of quercetin glycosides and water the poorest (Mikulic-Petkovsek et al., 2015a; Reis, Rai, & Abu-Ghannam, 2012). Elderberry spread and liqueur were extracted with a mixture of alcohol and water, which contributed to their higher flavonol levels. Contrary, tea and juice were not extracted with organic solutions, they were prepared just with water and the contents of flavonols in these products were lower compared to products containing organic solvents.

The group of hydroxycinnamic acids accounted less than 5% of all analyzed phenolics. Chlorogenic acid (*trans*-5-caffeoylquinic acid) was the major compound analyzed from this phenolic group. Elderberry liqueur, juice and spread were characterized by significantly highest levels of neochlorogenic acid (3-caffeoylquinic acid). However, the control treatment (46 mg/kg) and tea (39 mg/kg) contained highest levels of total hydroxycinnamic acids. A major contributor to total hydroxycinnamic acids in tea was *p*-coumaric acid hexoside, which was also abundant in spread. 3-*p*-coumaroylquinic acid and *p*-coumaric acid have additionally been detected in elderberry juice, although these two compounds could not be identified in tea. Other cinnamic acid derivatives were determined in smaller quantities in all analyzed elderberry products (the contents were lower than 2 mg/kg) (Table 1). Selected hydroxycinnamic acids (chlorogenic acid, 3-*p*-coumaroylquinic acid and different dicaffeoylquinic acids) were affected by thermal processing. Their degradation increased with higher temperatures and longer heating time, similar to anthocyanin turnover (Budryn, Nebesny, & Rachwal-Rosiak, 2013), with the difference hydroxycinnamic acids are more thermo stable (Zorić et al., 2014). In study of Zorić et al. (2014) reported that the greatest decrease of chlorogenic acid occurred at a temperature above 100 °C after 20–30 min. Chlorogenic acid in elderberry spread in our study greatly decreased compared to control, due to heat treating at boiling point for one an hour. Additionally, reactions of polyphenols with carbohydrate and lipid radicals as well as proteins through covalent interactions lead to an irreversible loss of

**Table 2**
The content of phenolic compounds and sambunigrin (cyanogenic glycoside) (mean ± standard error in mg/kg) in different elderberry products.

| Phenolics | Control | Juice | Liqueur | Tea | Spread |
|---|---|---|---|---|---|
| Chlorogenic acid (*trans*-5-caffeoylquinic acid) | 12.6 ± 2.3$^{a}$ | 0.63 ± 0.09$^{c}$ | 4.8 ± 0.9$^{b}$ | 7.0 ± 5.3$^{b}$ | 1.6 ± 0.3$^{c}$ |
| Neochlorogenic acid (3-caffeoylquinic acid) | 9.7 ± 5.6$^{b}$ | 6.8 ± 1.4$^{ab}$ | 12.2 ± 7.6$^{a}$ | 4.6 ± 2.1$^{b}$ | 8.2 ± 5.6$^{ab}$ |
| *p*-Coumaric acid | 3.47 ± 0.40$^{ab}$ | 6.66 ± 4.65$^{a}$ | 1.57 ± 0.22$^{b}$ | / | / |
| *p*-Coumaric acid hexoside | / | / | / | 16.6 ± 7.1$^{b}$ | 3.6 ± 1.2$^{a}$ |
| 3-*p*-Coumaoylquinic acid | 10.5 ± 0.5$^{a}$ | 0.16 ± 0.05$^{c}$ | 6.3 ± 0.58$^{b}$ | / | 0.20 ± 0.04$^{c}$ |
| 4-*p*-Coumaoylquinic acid | 0.81 ± 0.04$^{a}$ | 0.35 ± 0.21$^{c}$ | 0.49 ± 0.11$^{b}$ | / | 0.53 ± 0.18$^{b}$ |
| 5-*p*-Coumaoylquinic acid | 0.40 ± 0.15$^{b}$ | 0.09 ± 0.04$^{b}$ | 0.13 ± 0.07$^{b}$ | 2.1 ± 1.5$^{a}$ | 0.53 ± 0.25$^{b}$ |
| 4-Caffeoylquinic acid | 0.22 ± 0.05$^{c}$ | 1.2 ± 0.16$^{b}$ | 0.27 ± 0.02$^{c}$ | 1.1 ± 0.28$^{b}$ | 2.2 ± 0.48$^{a}$ |
| *cis*-5-Caffeoylquinic acid | 1.4 ± 0.79$^{b}$ | 0.84 ± 0.36$^{b}$ | 0.75 ± 0.06$^{b}$ | 1.2 ± 0.59$^{b}$ | 2.7 ± 0.60$^{a}$ |
| Dicaffeoylquinic acid 1 | 1.5 ± 0.25$^{a}$ | 0.10 ± 0.02$^{c}$ | 0.25 ± 0.06$^{b}$ | / | / |
| Dicaffeoylquinic acid 2 | 3.2 ± 0.29$^{a}$ | 0.11 ± 0.04$^{d}$ | 0.73 ± 0.02$^{c}$ | 1.7 ± 0.85$^{b}$ | 1.8 ± 0.61$^{b}$ |
| Dicaffeoylquinic acid 3 | 2.1 ± 0.21$^{a}$ | / | 0.53 ± 0.28$^{b}$ | 1.2 ± 0.09$^{ab}$ | 0.97 ± 0.25$^{ab}$ |
| **Total hydroxycinnamic acids** | **46.0 ± 6.8**$^{a}$ | **15.4 ± 2.5**$^{d}$ | **30.1 ± 6.1**$^{bc}$ | **38.7 ± 7.9**$^{ab}$ | **22.4 ± 6.0**$^{cd}$ |
| (+) Catechin | 22.1 ± 1.8$^{a}$ | 1.5 ± 0.4$^{d}$ | 10.3 ± 1.4$^{b}$ | / | 4.7 ± 0.5$^{c}$ |
| (−) Epicatechin | 18.0 ± 5.0$^{b}$ | 2.3 ± 0.57$^{c}$ | 16.4 ± 2.6$^{b}$ | / | 23.3 ± 3.1$^{a}$ |
| **Total flavanols** | **40.1 ± 6.1**$^{a}$ | **3.8 ± 0.9**$^{c}$ | **26.7 ± 3.7**$^{b}$ | **/** | **27.5 ± 3.9**$^{b}$ |
| Quercetin-3-*O*-glucoside | 13.6 ± 2.4$^{a}$ | 0.17 ± 0.06$^{d}$ | 5.1 ± 0.67$^{c}$ | 3.7 ± 0.57$^{c}$ | 9.6 ± 1.9$^{b}$ |
| Quercetin-3-*O*-rutinoside | 87.6 ± 13.8$^{a}$ | 2.2 ± 1.0$^{d}$ | 38.0 ± 7.50$^{c}$ | 27.3 ± 7.2$^{c}$ | 65.0 ± 10.5$^{b}$ |
| Quercetin-acetylglucoside | 3.4 ± 0.30$^{a}$ | 0.05 ± 0.02$^{c}$ | 0.19 ± 0.08$^{c}$ | / | 1.9 ± 0.17$^{b}$ |
| Quercetin-hexoside pentoside 1 | 2.8 ± 1.0$^{b}$ | 0.12 ± 0.05$^{c}$ | 0.98 ± 0.56$^{bc}$ | 0.62 ± 0.20$^{c}$ | 10.7 ± 3.8$^{a}$ |
| Quercetin-hexoside pentoside 2 | 2.4 ± 0.73$^{a}$ | 0.12 ± 0.49$^{c}$ | 1.25 ± 0.31$^{b}$ | / | 3.0 ± 1.4$^{a}$ |
| Kaempferol-3-rutinoside | 9.7 ± 1.2$^{a}$ | 0.10 ± 0.03$^{e}$ | 3.8 ± 0.73$^{c}$ | 1.3 ± 0.38$^{d}$ | 7.2 ± 1.2$^{b}$ |
| Isorhamnetin-3-rutinoside | 3.0 ± 0.43$^{a}$ | / | 1.0 ± 0.15$^{c}$ | / | 2.0 ± 0.27$^{b}$ |
| **Total flavonols** | **122.2 ± 13.2**$^{a}$ | **2.8 ± 1.0**$^{e}$ | **50.5 ± 8.5**$^{c}$ | **32.8 ± 16.7**$^{d}$ | **99.4 ± 12.8**$^{b}$ |
| Naringenin hexoside 1 | 0.20 ± 0.03$^{a}$ | / | 0.03 ± 0.01$^{b}$ | / | / |
| Naringenin hexoside 2 | 0.29 ± 0.03$^{b}$ | / | 0.07 ± 0.01$^{c}$ | / | 0.40 ± 0.09$^{a}$ |
| **Total flavanones** | **0.48 ± 0.02**$^{a}$ | **/** | **0.08 ± 0.04**$^{c}$ | **/** | **0.40 ± 0.09**$^{b}$ |
| Cyanidin-3-*O*-glucoside | 288.7 ± 45.8$^{a}$ | 42.0 ± 8.8$^{c}$ | 88.3 ± 13.2$^{b}$ | 5.3 ± 0.52$^{d}$ | 34.8 ± 10.9$^{c}$ |
| Cyanidin-3-*O*-rutinoside | 33.3 ± 5.3$^{a}$ | 4.8 ± 1.0$^{c}$ | 10.2 ± 1.5$^{b}$ | 1.2 ± 0.20$^{d}$ | 6.5 ± 1.06$^{c}$ |
| Cyanidin-3-*O*-sambubioside | 328.1 ± 52.1$^{a}$ | 47.8 ± 10.1$^{cd}$ | 100.4 ± 15.0$^{b}$ | 21.0 ± 8.8$^{d}$ | 76.8 ± 33.3$^{bc}$ |
| Cyanidin-3,5-*O*-diglucoside | 19.2 ± 4.1$^{a}$ | 0.10 ± 0.04$^{c}$ | 1.1 ± 0.22$^{c}$ | 0.86 ± 0.17$^{c}$ | 4.7 ± 1.1$^{b}$ |
| Cyanidin-3-sambubiosyl-5-glucoside | 101.0 ± 21.6$^{a}$ | 3.5 ± 1.6$^{c}$ | 34.4 ± 6.7$^{b}$ | 28.7 ± 15.5$^{b}$ | 46.6 ± 8.6$^{b}$ |
| **Total anthocyanins** | **732.9 ± 77.8**$^{a}$ | **98.2 ± 21.3**$^{c}$ | **237.5 ± 37.0**$^{b}$ | **78.0 ± 8.5**$^{c}$ | **195.5 ± 35.0**$^{b}$ |
| **Total analyzed phenolics** | **958 ± 62.2**$^{a}$ | **114 ± 16.2**$^{c}$ | **343 ± 45.4**$^{b}$ | **162 ± 113.4**$^{c}$ | **337 ± 78.0**$^{b}$ |
| **Cyanogenic glycosides** | | | | | |
| Sambunigrin | 18.8 ± 4.3$^{a}$ | 10.6 ± 0.7$^{b}$ | 0.8 ± 0.21$^{d}$ | 3.8 ± 1.7$^{c}$ | 0.8 ± 0.19$^{d}$ |

Different letters (a–d) in rows denote statistically significant differences in individual phenolic and sambunigrin levels among elderberry products by Duncan multiple range test ($p < 0.05$); $n = 7$.

hydroxycinnamic acids (Budryn et al., 2013). The content of selected hydroxycinnamic acids differed between the liqueur and spread but thermal processing only moderately affected the contents of hydroxycinnamic acids in elderberry tea. Enhanced solubility of these compounds in water likely caused high hydroxycinnamic acids levels in the tea (Reis et al., 2012). As in our study also in study of Reis et al. (2012) chlorogenic acid showed the highest values in extraction with higher presence of water. Additional impact on total hydroxycinnamic acids at different elderberry products had time of extraction. Total hydroxycinnamic acids increased with the duration of water extraction (Mikulic Petkovsek et al., 2015a). In our study for tea were elder berries the longest topped into water.

Catechin and epicatechin have been identified from the group of flavanols, representing 4% of total analyzed phenolics. Bravo (1998) reported that some flavanols are more heat stable and the results of the present study confirm these findings as the contents of epicatechin increased or remained similar after heating in elderberry liquer and spread. Contrary were with elderberry juice and tea. Elderberry juice contained the lowest contents of flavanols, what is in accordance with the study of Hollman and Arts (2000), where explained that commercial preparation of fruit juices, including pressing, crushing, storage of concentrated juice at room temperature and decolouration decrease its flavanol contents. Flavanols could not be detected in elderberry tea as these compounds are poorly soluble in water and are more soluble in organic solvents (Viñas, López-Erroz, Marín-Hernández, & Hernández-Córdoba, 2000).

Flavanones only represented up to 0.05% total analyzed phenolics (Table 1). Moreover, the levels of flavanones were below detection in elderberry tea and juice. Wilcox, Borradaile, and Huff (1999) reported that these compounds are soluble in organic solvents (alcohol) but not in water, which explains their negligible in elderberry products prepared from the water basis. Naringenin derivatives have been detected in elderberry liqueur and spread, as organic solvents have been used for sample preparation. Liqueur was shorter time and in lower levels of organic solvents exposed to heat treatment than spread. Cheig, Chung, & Chung (2012) reported that short extraction time (<10 min) above boiling may decrease the efficiency of flavanones because of their insufficient solubilization. However, only small amounts could be detected in these elderberry products (Table 2).

On the other hand, thermal processing increased the level of specific phenolic compounds such as neochlorogenic acid, 4-

caffeoylquinic acid, *cis*-5-caffeoylquinic acid, epicatechin, quercetin-hexoside pentoside and naringenin hexoside. This is in accordance with the study of Kim, Kang, and Gweon (2013), who determined that heat treatment destroys plant cell walls and releases bound of selected phenolic compounds. Because of different chemical structures their contents of phenolics differ during processing into products. Higher temperature can promote higher analyte solubility by increasing solubility and mass transfer rate. On the other hand, with higher temperature viscosity and the surface tension of solvents decreased, which helps the solvents to reach the sample matrices and improving the extraction rate. Many phenolics are easily hydrolyzed and oxidized. Long extraction time and high temperature increase the chance of oxidation, which decrease the yield of phenolics in extracts (Dai & Mumper, 2010). The longest thermal processing has been recorded for elderberry spread, which triggered an increase of selected phenolics in the samples: 4-caffeoylquinic acid (10 fold increase compared to the control) and *cis*-5-caffeoylquinic acid among hydroxycinnamic acids, epicatechin among flavanols, quercetin-3-*O*-xyloside, quercetin-hexoside pentoside 1 (5 fold increase compared to the control) and quercetin-hexoside pentoside 2 among flavonols and naringenin hexoside 2 among flavanones. However, cell wall degradation is not the only reason for increased levels of selected compounds in elderberry spread. Heating deactivates endogenous oxidative enzymes resulting in prevention of enzymatic oxidation and consequently a loss of selected antioxidant compounds (Choi et al., 2006). Peleg, Naim, Rouseff, and Zehavi (1991) uncovered an additional effect of thermal processing and reported a potential formation of novel compounds with antioxidant activity during exposure of plant samples to heat. Lately, Kim et al. (2013) defined that longer heating at lower temperatures stimulate the increase of compounds compared to higher temperatures or boiling for shorter periods.

Harmful cyanogenic glycosides have been quantified in addition to individual phenolics. Sambunigrin was the only cyanogenic glycoside detected in all analyzed elderberry products. Its content was much lower than the level of phenolics and ranged from 0.8 (elderberry liqueur) to 11 mg/kg (elderberry juice) (Table 2). Highest levels of sambunigrin have been measured in fresh elder berries (control sample) (19 mg/kg) and a 44% decrease of this cyanogenic glycoside has been recorded in elderberry juice, which contained highest levels of sambunigrin of all analyzed elderberry products. Juice was relatively concentrated, which contributed to its higher sambunigrin content compared to other products. A moderate content of sambunigrin has been measured in elderberry tea (4 mg/kg). Onyeike and Omubo-Dede (2002), Akande and Fabiyi (2010) and Kawamura, Hikidi, Maruyama, Uchiyama, and Saito (1993) reported that cooking or boiling reduces the content of cyanogenic glycosides by 51%–67%. Boiling water could not completely reduce the toxic effects of sambunigrin in elderberry tea. Moreover, as dried elderberries were used for the infusion the initial level of cyanogenic glycosides was much higher compared to fresh fruit.

The lowest contents of the cyanogenic glycoside in our study have been measured in elderberry liqueur and spread (0.8 mg of sambunigrin per kg) and a 96% reduction of sambunigrin levels has been recorded in these two products compared to the control treatment. Obviously, heat treatment significantly diminished the levels of cyanogenic glycosides in elderberry liqueur and spread. Higher temperatures in elderberry spread preparation effectively degraded cyanogenic glycosides and the dilution with water and alcohol caused lower levels of sambunigrin in elderberry liqueur. In study of Akande and Fabiyi (2010) reported that cooking for 60 min was enough for elimination of the most thermo-labile compounds, such as cyanogenic glycosides and alkaloids. Nevertheless, some previous studies reported that boiling process was not completely effective in reducing the levels of cyanogenic glycosides. Kawamura et al., (1993) determined that the maximum decrease occurred when we soaking and refining food products rich in cyanogenic glycosides. Akande and Fabiyi (2010) disagree with previous statement. Soaking could be effective only with the discarded soaking solution and some cyanogenic glycosides (prunasin and amygdalin) are partly hydrosoluble compounds. Authors of this study reported that autoclaving and two stage-cooking, wherein after first cooking discarding the initial water used and for second cooking poured fresh water generate the highest elimination of some toxic compounds.

Although elderberry liqueur contained highest levels of phenolics (Fig. 1) the highest content of cyanogenic glycosides was retained in the juice, which was characterized by 14 fold higher levels as measured in elderberry spread and liqueur. Bermúdez-Soto and Tomás-Barberán (2004) recorded that elderberry juice is rich in total phenolics, anthocyanins and flavonols but the results of our study also mark it as a high source of harmful components. 0.01 mg cyanogenic glycosides and 44.9 mg of phenolics are introduced into the body if a single cup of elderberry tea is consumed (data not shown). Much smaller quantities (0.003 mg cyanogenic glycosides and 91.4 mg of phenolics) are associated with the intake of the liqueur as it is usually considered as a shot drink (50 mL). Finally, a serving of elderberry juice (200 mL) contains 0.03 mg cyanogenic glycosides and 58 mg phenolics. Mills and Bone (2005) reported that selected elderberry products are safe if taken in moderation and that the results of the present study confirm these findings. All processed products were namely characterized by lower phenolic and cyanogenic levels than the control berries, as their manufacture included thermal treatment. Bolarinwa, Orfila, and Morgan (2014) reported, that the levels of cyanogenic glycosides in range of 0.04–1.2 μg/g (detected in processed products) are not likely to give rise to any toxicity concerns. In this aspect elderberry beverages do not present any toxicity issues. It has been confirmed that pasteurization affectively decreases the levels of harmful compounds, such as cyanogenic glycosides. On the other hand, the stability and degradation of selected phenolics, mainly anthocyanins, are affected by any process, which includes heating. Among the analyzed elderberry products, liqueur and spread are superior sources of phenolics and also contain lowest levels of harmful sambunigrin. Contrary, elderberry juice and tea retained relatively high levels of cyanogenic glycosides and only low amounts of phenolic compounds. Diverse levels of sambunigrin and phenolics determined in our analysis compared to data reported in other studies can be linked to different fruit maturity stages. Zahmanov, Alipieva, Simova, and Georgiev (2015) reported, that mature elderberry contains lower levels of cyanogenic glycosides than immature fruit and leaves, which has been linked to different catabolism of plant tissues. Moreover, the accumulated aliphatic and aromatic molecules decompose with the progression of fruit maturity and the products are used as building blocks to produce (predominantly) sugars (Zahmanov et al., 2015). In accordance with this study, elderberry products evaluated in the present analysis contained exceptionally low contents of cyanogenic glycosides as they were all produced from fully mature fruits.

## 4. Conclusion

Thermal processing, time and type of extraction solution greatly affected phenolics and cyanogenic glycosides in different elderberry products. Their contents varied depending of their processing method. Ultimately, processing significantly decreases the content of harmful cyanogenic glycosides, but also affects beneficial

anthocyanins. Preparation of elderberry products, which include processing at high temperatures, does not always contribute to their inferior quality, as high temperature can effectively reduce the levels of harmful components and make elderberry products safer to consume.

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